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ABSTRACT

The possible role of histone deacetylase inhibitors (HDACi) in breast cancer treatment is an area of active investigation. However, its potential as a preventive agent has not been studied. Valproic acid (VPA) is an HDACi which has been used for many decades to safely treat neurological disorders. The rationale for the use of HDACi in breast cancer prevention is a previously unexplored area of research that is based on compelling preclinical data. Epidemiologic studies showing an association between HDACi use and breast cancer incidence would be important evidence to support future prospective clinical trials of HDACi in cancer prevention. **We sought to ascertain whether the risk of incident breast cancer is reduced in patients with a history of VPA use, and if so, to determine whether this effect is proportional to the duration of VPA use and whether all breast cancer subtypes are impacted similarly.** We developed a database using de-identified data from the Kaiser Permanente of Northern California (KPNC) clinical and pharmacy records of members of the KPNC Healthplan between 1997 and 2007. 20,864 breast cancer cases and 208,640 controls matched for birth year and duration of KNPC pharmacy coverage were identified. 68 incident breast cancers were seen among women with history of VPA use; 486 were in women without history of VPA use. Mean age at diagnosis of the cohort was 61.8 years; mean years of prescription drug coverage was 7.4 years. Among cases, 73% of the cohort was non-hispanic white, 7.6% were African American, and 10.5% were Asian/Pacific Islander. When compared to never users, patients with at least 2 years of VPA use had an increased odds of a breast cancer diagnosis (OR 1.37; 95% CI 1.06-1.76). This effect was only significant for HR-positive incident tumors, although the numbers of HR-negative cases was small (n=12). These findings support that VPA use is not associated with reduction of breast cancer incidence. Moreover, we cannot exclude the possibility that VPA may be associated with a small but increased risk of HR-positive breast cancer. HDAC inhibition does not appear to be an effective or tractable strategy for breast cancer prevention.

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INTRODUCTION

The possible role of histone deacetylase inhibitors (HDACi) in breast cancer treatment is an area of active investigation. However, its potential as a preventive agent has not been studied. Valproic acid (VPA) is a commonly prescribed HDACi which has been used for many decades to safely treat a wide variety of neurological disorders. The rationale for the use of HDACi in breast cancer prevention is a previously unexplored area of research based on compelling preclinical data that shows VPA reduces risk of invasive breast cancer in animal models. Epidemiologic studies showing an association between HDACi use and breast cancer incidence would be important evidence to support future prospective clinical trials of HDACi in cancer prevention. **We hypothesize that the risk of incident breast cancer is reduced in patients with a history of VPA use, and that this effect is proportional to the duration of VPA use. The Specific Aims we plan to achieve are the following: Aim 1:** We will compare the incident breast cancer rate in women with a history of valproic acid use to an age-matched cohort without VPA use, adjusting for potential confounders. We will establish whether VPA is associated with a reduced risk of breast cancer in this cohort, and whether duration of therapy impacts this risk. **Aim 2:** We will determine whether the association between VPA use and incident breast cancer differs among patient populations and tumor subtypes. If feasible, we will examine this association among different race/ethnicities as well as evaluate the tumor characteristics associated with VPA use.

BODY

Background. Recent emergence of the role of epigenetic regulation in cancer progression has resulted in epigenomic targeting as an important focus of investigation into treatment and prevention strategies[1, 2]. Among these epigenetic alterations are mechanisms affecting histone acetylation. Acetylation allows for neutralization of positively charged histones resulting in a conformational change of chromatin. Accessibility of transcription factors and promoter regions of DNA permits active gene expression[3]. It has been shown that aberrantly hypoacetylated histone complexes combine with DNA methylation to effect gene silencing, a prominent feature of carcinogenesis. Previous work has shown significant reduction in histone acetylation from normal epithelium to ductal carcinoma in situ (DCIS) suggesting that this is an early event in the cancer pathway[4]. Cell culture data support that HDAC inhibition induces apoptosis in a wide variety of tumors[5-8]. Further, drugs targeting aberrant deacetylation (histone deacetylase inhibitors--HDACi) have been shown to down-regulate estrogen receptor α accumulation in breast cancer cells[9, 10]. Thus, targeting of abnormal histone deacetylation by a class of drugs by HDACi may be a possible therapeutic and/or preventive strategy for breast cancer[11].

One well-known HDACi, valproic acid (VPA), has been used safely for decades primarily for neurologic indications including epilepsy, bipolar disorder, clinical depression, and migraines. In preclinical studies, VPA has been shown to reduce cancer cell proliferation in breast cancer cell lines, and promotes differentiation of cancer cells. Furthermore, when combined with tamoxifen, proliferation is inhibited to a greater degree than with either VPA or tamoxifen alone, suggesting the presence of a synergistic relationship[12]. However, few studies have explored whether there exists an association between VPA use in humans and breast cancer incidence. Epidemiologic studies showing an association between HDACi use and breast cancer incidence would be important evidence to support future clinical trials of HDACi in cancer prevention. We undertook this study to determine whether a history of VPA use in a managed care patient cohort could impact both incidence and cancer phenotype when compared to controls without VPA exposure.

Methods. The Kaiser Permanente Medical Care Program (KPMCP) of Northern California is a managed health care system serving roughly 30% of residents in the San Francisco Bay area and the Central Valley. Except for some under-representation from either end of the socioeconomic spectrum, membership is representative of the local population with about half of the membership being female[13]. Sources of data for this study come from two cohorts of members; Kaiser members with a history of VPA use and a control group without history of VPA use.

The Pharmacy Information Management System (PIMS) within KPMCP records all outpatient medication dispensed to Kaiser members through any Kaiser pharmacy. As an integrated health care system, nearly 100% of members fill all their prescriptions at KPMCP pharmacies[14]. All KPMCP pharmacies began to implement PIMS in August 1994. Follow-up began from enrollment into KPMCP with drug coverage, with PIMS refill history used to estimate continuity of use and duration of use. Unless valproic acid was dispensed after August 1994, all women were considered to be nonusers. Records of valproic acid dispensing were obtained through December 31, 2008, with a minimum of one year of medication data for each subject required. Women obtaining drug coverage after December 31, 2008 were excluded.

Incidence of carcinoma in situ and invasive breast cancer were obtained through KPMCP's Cancer registry. Ascertainment of carcinoma in situ and invasive breast cancer occurrence were recorded from January 1, 1997 to December 31, 2007. Women with a diagnosis of breast cancer prior to the study period were excluded. For all subjects, follow-up ended with breast cancer diagnosis, termination of KPMCP coverage, death, or on December 31, 2008, whichever came first.

Analysis was performed using a nested case-control approach. For each patient with a history of VPA use, ten control patients with no history of VPA use were randomly selected from the cohort. Controls were matched with subjects for year of birth and year of starting drug coverage.

Results

20,864 cases diagnosed with invasive breast cancer between 1997 and 2007 and 208,640 controls were identified. Characteristics of the cases, controls, hormone receptor (HR)-negative cases and HR-positive cases were compared (Table 1). The KPMCP patient population is predominantly non-Hispanic white (73.2%); 7.6% and 10.5% of cases were of African American and Asian/Pacific Islander descent respectively. There were no significant differences in years of prescription drug coverage between cases and controls, with 7.4 median years of coverage in both groups. As expected, exogenous hormone therapy use was greater in cases than controls, an association which was limited to the HR-positive tumors. HR-negative breast cancers constituted 18% of cases (n=3669). 99.2% of the cohort had never had a history of VPA prescription use. Among past and current users of VPA, 30% had at least 2 years of use. Only 68 cases of incident breast cancer (12 HR-negative and 56 HR-positive) had at least 2 years of VPA use.

The association between duration of VPA use and odds of a new breast cancer diagnosis was evaluated and adjusted for age at diagnosis and years of prescription drug coverage (Table 2). Compared to never users, women with at least 2 years of VPA use had a small but

significant increase in risk of breast cancer (OR 1.37, 95% CI 1.06-1.76). Overall, increased duration of VPA use was associated with greater odds of developing breast cancer, although this trend was not significant. This association was further explored in an analysis stratified by HR receptor status (Table 3). The small number of cases precluded a robust analysis for the ER-positive cancers. However, the increased risk with at least 2 years of VPA use was seen in the ER-positive tumors. Again, there appeared to be increased risk for ER-positive tumors with increased duration of use; this association was not seen in ER-negative tumors.

Table 1. Characteristics of breast cancer cases diagnosed during 1997-2007 at KPNC and matched controls, by disease and hormone receptor status.

Characteristic	All Controls N=208,640	All Cases N=20,864	HR-negative Cases N=3,669	HR-positive Cases N=17,195
Age at diagnosis/index date (years): mean ± s.d.	61.88 +/- 13.00	61.76 +/- 13.00	58.59 +/- 13.24	62.65 +/- 12.84
Years of prescription drug coverage: mean ± s.d.	7.43 +/- 3.18	7.43 +/- 3.18	7.37 +/- 3.15	7.44 +/- 3.19
***Oral contraceptive use:** n, (col%)	13,492 (6.5)	1,618 (7.7)	335 (9.1)	1,283 (7.5)
†Menopausal hormone therapy use: n, (col%)	68,347 (32.8)	7,673 (36.8)	1,203 (32.8)	6,470 (37.6)
Valproic Med use: n, (col%)				
Never use	207,034 (99.2)	20,697 (99.2)	3,642 (99.3)	17,055 (99.2)
"Ever" use	1,606 (0.8)	167 (0.8)	27 (0.7)	140 (0.8)
"≥ 2 years" use	486 (0.2)	68 (0.3)	12 (0.3)	56 (0.3)
Years of Valproic Med use: mean ± s.d.				
Among "Ever" users	1.46 ± 2.19	1.81 ± 2.59	1.45 ± 2.23	1.90 ± 2.68
Among "≥ 2 years" users	4.71 ± 2.59	4.91 ± 2.86	4.74 ± 2.02	4.95 ± 3.02
Race/Ethnicity: n, (col%)				
Non-Hispanic White	n/a	15,283 (73.2)	2,322 (63.3)	12,961 (75.4)
Hispanic White	n/a	1,386 (6.6)	318 (8.7)	1,068 (6.2)
African American	n/a	1,597 (7.6)	517 (14.1)	1,080 (6.3)
Asian/Pacific Islander	n/a	2,193 (10.5)	419 (11.4)	1,774 (10.3)
Other	n/a	405 (1.9)	93 (2.5)	312 (1.8)

* Oral contraceptive "ever" use within 10 years prior to diagnosis/index date
† Hormone therapy "ever" use within 5 years prior to diagnosis/index date

Table 2. Risk of breast cancer associated with Valproic Med use

Valproic Use	Cases N=20,864 n (col%)	*Matched Controls N=208,640 n (col%)	†OR (95% CI)
Never	20,697 (99.2)	207,034 (99.2)	**Reference**
≥ 2 years	68 (0.3)	486 (0.2)	**1.37 (1.06-1.76)**
Never	20,697 (99.2)	207,034 (99.2)	**Reference**
< 1 yr	74 (0.3)	849 (0.4)	**0.84 (0.66-1.07)**
≥ 1 – 2 yrs	25 (0.1)	271 (0.1)	**0.90 (0.60-1.36)**
≥ 2 – 3 yrs	18 (0.1)	154 (0.1)	**1.14 (0.70-1.86)**
≥ 3 – 5 yrs	27 (0.1)	166 (0.1)	**1.60 (1.07-2.41)**
≥ 5 yr	23 (0.1)	166 (0.1)	**1.35(0. 87-2.09)**

* Controls matched to cases based on age at diagnosis/index date and years of prescription drug coverage
† Odds ratios based on conditional logistic regression among cases and their matched controls, adjusted for OC and HT use

Table 3. Risk of HR negative or positive breast cancer associated with Valproic Med use

Valproic Use	HR-negative Cases N=3,669 n (col%)	*Matched Controls N=36,690 n (col%)	†OR (95% CI)	HR-positive Cases N=17,195 n (col%)	‡Matched Controls N=171,950 n (col%)	§OR (95% CI)
Never	3,642 (99.3)	36,391 (99.2)	**Reference**	17,055 (99.2)	170,643 (99.2)	**Reference**
≥ 2 years	12 (0.3)	88 (0.2)	**1.34 (0.73-2.45)**	56 (0.3)	398 (0.2)	**1.38 (1.04-1.82)**
Never	3,642 (99.3)	36,391 (99.2)	**Reference**	17,055 (99.2)	170,643 (99.2)	**Reference**
< 1 yr	14(0.4)	162 (0.4)	**0.84 (0.49-1.46)**	60 (0.3)	687 (0.4)	**0.84 (0.65-1.10)**
≥ 1 – 2 yrs	1 (0.03)	49 (0.1)	**0.20 (0.03-1.48)**	24 (0.1)	222 (0.1)	**1.05 (0.69-1.60)**
≥ 2 – 3 yrs	3 (0.1)	37 (0.1)	**0.80 (0.24-2.59)**	15 (0.1)	117 (0.1)	**1.25 (0.73-2.14)**
≥ 3 – 5 yrs	4 (0.1)	26 (0.1)	**1.54 (0.54-4.41)**	23(0.1)	140 (0.1)	**1.62 (1.04-2.52)**
≥ 5 yr	5 (0.1)	25 (0.1)	**1.95 (0.75-5.11)**	18 (0.1)	141 (0.1)	**1.24 (0.76-2.03)**

* Controls matched to HR-negative cases bases on age at diagnosis/index date and years of prescription drug coverage
† Odds ratios based on conditional logistic regression among HR-negative cases and their matched controls, adjusted for OC and HT use
‡ Controls matched to HR-positive cases bases on age at diagnosis/index date and years of prescription drug coverage
§ Odds ratios based on conditional logistic regression among HR-positive cases and their matched controls, adjusted for OC and HT use

Discussion

In this case-control study of incident breast cancer in a managed care cohort, we did not see an association between valproate use and incidence of invasive breast cancer, either in ever-users or those with at least 2 years of valproic acid use. There has been one previous epidemiologic study of VPA use and incident cancers in the Danish Cancer Registry[15]. This study showed a suggestion that breast cancer incidence may be reduced in women with VPA use, although the result was not statistically significant (0.62; 95%CI 0.21-1.76). However, the number of cases was small (4 exposed cases, 26 exposed controls), precluding subset analysis by hormone receptor status. In the current larger study, 68 cases had at least 2 years of VPA use; subset analysis by HR status did not show an effect of VPA on either HR-negative or HR-positive breast cancer incidence.

Substantial preclinical data have shown that VPA is an active agent against numerous cancer cell lines[5, 7, 12, 16]. This effect has been validated in animal models of breast cancer[12]. Multiple reasons may explain why there was no effect seen in human epidemiologic studies. These include inability to control dose and duration of drug use, and lack of data to indicate whether a threshold level of exposure to VPA is required to demonstrate an effect. Although it is unknown whether the duration of use could impact efficacy of VPA, previous studies of tamoxifen use in both the primary and secondary prevention settings suggest that there is a significant risk reduction by two years of use, and no such effect was seen in this cohort. Since there is no evidence to suggest that women with neurologic diagnoses amenable to VPA treatment have a reduced risk of breast cancer, and since there is no trend of lower breast cancer rate with duration of use, one must conclude that there appears to be no preventive effect in breast cancer incidence with HDAC inhibition.

The strength of this study lies in the large Northern California Kaiser cancer database in which over 208,000 incident breast cancers were identified between 1997 and 2007. This registry is electronically linked to a closed pharmacy system through which all members are required to have prescriptions filled to obtain drug coverage. This allows for accurate central tracking of all filled prescriptions, including dose and duration of use. The social and racial diversity within this managed care population increases the generalizability of the findings.

Despite this considerable resource however, there were still few patients who had both a history of VPA use and documentation of an incident breast cancer diagnosis. It is unknown whether a larger dataset may be able to yield additional insight, but since there are few databases which are sufficiently large and robust to permit this type of analysis, it is doubtful whether another data source could be used to either validate or refute these findings. Importantly, we cannot exclude that VPA may in fact be associated with a small but significant increase in breast cancer risk (OR 1.37), particularly for HR-positive breast cancer.

We conclude that in a cohort of 20,864 breast cancer cases and 208,640 controls matched for age at diagnosis and year of prescription drug coverage, there was no reduction in breast cancer incidence with VPA use. A small increased risk with VPA use cannot be excluded. These findings support that HDAC inhibition is not likely to be an effective mechanism for breast cancer prevention.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

- Identification of cases and controls: Cases status was determined as those female members identified by the KPNC Cancer Registry as having a diagnosis of invasive breast cancer with known ER status between 1997 and 2007.

- Cases were matched to controls on the basis of year of birth and duration of KPNC pharmacy coverage. 22,488 breast cancer cases and 224,960 controls have been thus identified. Among cases, 3,996 cases were found to be ER-negative, and 18,492 were ER-positive.

- VPA formulations carried by the KPNC pharmacy were identified and consisted of valproic acid, valproate sodium, and divalproex sodium. ICD-9 codes for indications for use have also been identified: epilepsy/seizure disorder (345.0-345.9/780.39), depression (296.2, 296.3, 311), and migraine (346.0=346.9).

- Use of exogenous hormones in this population has also been collected in the database.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

A summary of the results above showing an absence of an association between VPA use and breast cancer incidence has been prepared for manuscript submission.

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

In a managed care cohort with outpatient pharmacy records and cancer incidence/outcome tracking, no association between valproic acid use and breast cancer incidence could be identified. Although preclinical data shows some potential for VPA in cancer prevention, this does not appear to be reflected in reduction of either ER-positive or ER-negative breast cancer.

Facilities and Resources
Primary data retrieval was performed at the Division of Research at Northern California Kaiser. All other study-related activities were conducted at the UCSF Comprehensive Cancer Center, which houses the research staff for the UCSF Breast Care Center.

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